Title

Enriched on-grown *Artemia* metanauplii actively metabolise highly unsaturated fatty acid-rich phospholipids

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Abstract

On-grown (metanaupliar) stages of *Artemia*, have been regarded as more adequate preys for early life-cycle stages of cephalopods, crustaceans, and a variety of fish species. In recent studies, we obtained successful enhancements of highly unsaturated fatty acids (HUFA) and polar lipids (PL) in enriched *Artemia* metanauplii using either a combination of a commercial, neutral lipid (NL)-based HUFA-rich emulsion and Soya lecithin, or HUFA-rich phospholipids. The present study aimed at exploring the molecular form under which dietary HUFA are actually deposited in the metanaupliar lipids. Thus, we analysed the fatty acid (FA) composition of the PL and NL fractions from enriched metanauplii, with special emphasis to the fate of docosahexaenoic acid (DHA) within *Artemia* lipids. The results show that on-grown *Artemia* actively translocated ingested FA contained into PL to NL classes including triacylglycerides.

1. Introduction

Highly unsaturated fatty acids (HUFA) and polar lipids (PL) are regarded as essential nutrients for marine species and beneficial aspects derived from the dietary intake of these compounds have been reported on survival, growth, normal development and stress tolerance (Cahu et al., 2009; Glencross, 2009; Kanazawa, 1997; Sargent et al., 1997; Tocher et al., 2008; Tocher, 2010). For example, dietary intake of the HUFA docosahexaenoic acid (22:6n-3, DHA) is required for early life-cycle stages of marine finfish, since they have an apparently limited ability for endogenous biosynthesis of this essential nutrient (Bell et al., 2003; Tocher, 2010). On the other hand, phospholipids, a predominant fraction among PL, have emulsifying properties (Koven et al., 1993; Olsen et al., 1991) that may facilitate lipid absorption and increase the tolerance to stress conditions (Kanazawa, 1997). Importantly, HUFA delivered as PL have more beneficial effects than those delivered as neutral lipids (NL) (Cahu et al. 2003; Cahu et al. 2009; Gisbert et al., 2005; Rainuzzo et al., 1994). Additionally, live preys as copepods naturally contain high levels of essential HUFA, like eicosapentaenoic acid (20:5n-3, EPA) and DHA, predominantly esterified into phospholipids (Bell et al., 2003).

Enrichment protocols have been developed to enhance the nutritional quality of live preys used in aquatic larviculture (Conceição et al., 2010). *Artemia*, particularly its newly hatched naupliar stages, is arguably the most commonly used live prey in marine finfish and crustacean larviculture (Conceição et al., 2010; Sorgeloos et al., 2001). However, their suitability as a diet for marine larvae has been often questioned due to their relatively low HUFA and PL contents in comparison with natural preys such as copepods (Bell et al., 2003). Extensive investigations have been carried out on this subject, but it is still difficult to enrich live preys such *Artemia* with adequate levels of essential HUFA, particularly DHA (Tocher et al. 2010). DHA contents of enriched *Artemia* have been reported as unstable by different authors (Evjemo et al. 1997, Triantaphyllidis et al. 1995), since undesired metabolic conversions of lipid classes containing DHA (Harel et al., 1999; McEvoy et al., 1996; Rainuzzo et al., 1994) and from DHA to other FA (Navarro et al., 1999) may occur. Consequently, establishing

optimised protocols for the simultaneous bioencapsulation of HUFA-rich PL into *Artemia* is a challenge that needs to be urgently achieved.

On-grown stages of *Artemia*, namely metanauplii, are live preys less commonly used than nauplii, but regarded as having more adequate size for feeding early life-cycle stages of some organisms like cephalopods (Domingues et al. 2001; Iglesias et al., 2006), crustaceans (Ritar et al., 2002) and a variety of fish species (Lim et al., 2003, Woods, 2003; Zaki and Saad, 2010). Information about the use of metanauplii or *Artemia* biomass as live preys is scarce as compared to that on the use of newly hatched nauplii. In a recent study, we succeeded in the simultaneous enhancement of HUFA and PL contents of *Artemia* metanauplii (Guinot et al., 2013). Two different enrichment diets were used: 1) a combination of soya lecithin (SL) and the commercial emulsion Easy DHA Selco (containing HUFA-rich NL); and 2) the commercial product Marine lecithin LC60 (ML), a HUFA-rich PL-based product with great potential as enrichment diet (Guinot et al., 2013). Beyond the goal of this former approach aiming at establishing optimised enrichment protocols, the present study focused at exploring the molecular form under which dietary HUFA were actually deposited in the metanaupliar lipids. We hereby show the FA compositions of the PL and NL fractions of the enriched metanauplii, with especial emphasis to the fate of DHA within *Artemia* lipids.

2. Materials and methods

2.1 *Artemia hatching and culture*

Artemia franciscana metanauplii were obtained from the hatching of Great Salt Lake cysts (INVE Aquaculture Nutrition, Dendermonde, Belgium). Cysts were incubated during 24 h in 1 L cylinder-conical glass tubes containing seawater (37 g L^{-1} salinity) at 28°C, continuous light and vigorous aeration. After hatching, Instar I nauplii were placed in seawater at room temperature in 90 L cylindrical methacrylate containers, at a density of 4000 individuals L^{-1}. Nauplii were fed daily microalgae *Tetraselmis suecica* at densities around 200000 cells mL^{-1}. *Artemia* metanauplii were grown for 5 days,

attaining a mean length of 1.5 mm and subsequently used in the different enrichments procedures.

2.2 ***Composition of products used in enrichment diets***

Soya lecithin (SL, Korot SL, Alcoy, Spain) contained 74 % total lipids, mainly (80%) as PL, 52 % of total FA as linoleic acid (18:2n-6, LA) and lacked EPA and DHA. Easy DHA Selco (SS) contained 18 % DHA of total FA presented largely as NL. Marine lecithin LC60 (ML, PhosphoTech Laboratories, St. Herblain, France) contained 68 % total lipids (~50 % of TL being PL), with 13 % and 33 % of total FA as EPA and DHA, respectively. Soya and marine lecithin were dispersed in seawater with a domestic blender and Easy DHA Selco was self-dispersed following supplier's instructions. FA of total lipids, PL and NL of Soya lecithin, Easy DHA Selco and Marine lecithin LC60, are shown in Table 1.

2.3 ***Artemia metanauplii enrichments***

Two (triplicated) enrichment treatments were established: Treatment 1 (termed as 'Treatment 3C' by Guinot et al., 2013) consisted of a mixture of dispersed soya lecithin (0.3 g L^{-1}) and Easy DHA Selco (0.3 g L^{-1}); Treatment 2 consisted of a dispersion of Marine lecithin LC60 at 0.6 g L^{-1} (termed as 'Treatment 3A' by Guinot et al., 2013). The enrichment diets were dispensed at the beginning of the incubation and maintained for 4 h. The enrichment experiments were carried out by placing ~30000 five days old metanauplii in 0.5 L of filtered seawater at 28°C, vigorous aeration and continuous light. Metanauplii samples were collected and immediately stored at -80 °C for further analyses.

2.4 ***Analysis of fatty acids from Artemia total, polar and neutral lipids***

Total lipids from lyophilised *Artemia* samples were extracted according to the method of Folch et al. (1957) with the modifications described by Monroig et al. (2006). Two milligrams of total lipids were applied onto 20x20 cm Silica Gel 20 (Merck, Darmstadt, Germany) thin-layer chromatography (TLC) plates and subsequently eluted with a mixture of n-hexane:diethyl ether:acetic acid (85 :15: 1.5, v/v/v). One single PL fraction and two distinct NL fractions corresponding to triacylglycerides (TAG), and the

combination of monoacylglycerides, diacylglycerides and free fatty acids (hereafter referred to as 'combined fraction', CF) were scraped from the plate after identification and quantification (Olsen and Henderson, 1989) with known standards. FA methyl esters (FAME) from total, polar and neutral (TAG and CF) lipids were prepared by direct acid transmethylation following the protocols described in Christie (2003). FAME were analysed with a Thermo gas chromatograph (Thermo Trace GC Ultra, Thermo Electron Corporation, Waltham, MA, USA) fitted with an on-column injection system and a FID detector. Analytical temperature was programmed from 50 ºC to 220 °C. Chromatograms were integrated and analysed with Azur Datlys (St Martin d'Heres, France) software. FA were identified by comparison of retention times of each peak with those of well characterised standards.

2.5 ***Statistical analysis***

Statistical analyses were performed with the SPSS for Windows 15.0 statistical package (SPSS Inc., Chicago, IL, USA). Data are expressed as means ± standard deviations (n=3). The FA profiles obtained were integrated chemometrically in a principal component analysis (PCA) model. The score plot obtained after the generation of the two principal components was used to identify patterns of distribution of FA among treatments and lipid classes.

3. Results

Total lipids from Treatment 1 metanauplii accounted for 16.5 ± 1.0 mg g dw^{-1}. Quantification of the different lipid fractions prepared from *Artemia* metanauplii samples showed that PL accounted for 30 % of total lipids, whereas NL accounted for 70 % (25% TAG and 16% CF) of total lipids in both treatments. FA composition of total lipids, PL and both NL fractions (TAG and CF) from Treatment 1 *Artemia* is shown in Table 2. The main FA found in total lipids included 16:0, 18:0, 18:1, 18:2n-6, 18:3n-3, 22:5n-3 and 22:6n-3. DHA (22:6n-3), supplied in the enrichment diet of Treatment 1 mainly as NL (Table 1), reached up to 5.8 % in the total lipid fraction of metanauplii. While DHA represented only 0.8 % of total FA in PL, DHA contents of

12.7 % in TAG and 10.4 % in CF were found, indicating that it was mainly deposited into *Artemia* NL. Importantly, LA (18:2n-6), the main FA of Soya lecithin (Table 1), accounted for 6.8 % of total FA in PL, 10.9 % as TAG and 14.1 % in CF.

In Treatment 2, *Artemia* metanauplii contained 17.6 ± 0.1 mg g dw^{-1} total lipids. FA composition of total lipids, PL and both NL fractions from Treatment 2 metanauplii are presented in Table 3. The main FA found in the total lipid fraction were 16:0, 18:0, 18:1, 18:3n-3, 20:5n-3 and DHA. Content of DHA, presented basically as PL in the enrichment diet Marine lecithin LC60 (Table 1), reached a value of 13.1 % in the total lipid fraction of *Artemia* metanauplii. Interestingly, only 1.9 % of total FA in the metanaupliar PL were as DHA, where the contents of this fatty acid attained 26.0 % of total FA in TAG and 22.2 % in CF. Clearly, this result showed again a preferential deposition of DHA in the NL of enriched *Artemia*.

For the sake of comparison, the FA profile of TL, PL and NL of unenriched metanauplii are presented in Table 4. Fractionation an chromatographic analysis allowed an estimation from the chromatographic signal of more than 90 % of the FA from TL being esterified in PL, with TAG and CF showing chromatograms with a very weak response and small number of peaks. Thus, only PL and TL FA from unenriched nauplii were introduced in the chemometric analysis.

The chemometric integration of the FA profiles as variables in a PCA model (Fig. 1A) revealed that the first component (PC1) accounted for 45 % of the variance, and grouped the FA associated with *Artemia* intrinsic composition (18:0, 18:4n-3, 18:3n-3, 20:0, 20:3n-3 and 22:0) on the positive side of the axis, whereas DHA loaded on the negative side. Component 2 (PC2) further explained 27 % of the variance and was associated with 16:1n-7, 18:2n-6, 20:4n-3 and 22:1n-11 on the positive side. The results of the factor score plot (Fig. 1B) clearly show differential distribution of the FA patterns of the lipid classes, with three distinctive groups corresponding to PL, NL and total lipids of enriched *Artemia* metanauplii. PL profiles, grouped on the positive side of PC1 (showing association to 18:0, 18:4n-3, 18:3n-3, 20:3n-3 and 22:0), presented low dispersion, and were remarkably similar between treatments. NL profiles, grouped on

the opposite side of PC1, were also partly (Treatment 1) associated to the positive side of PC2 and the corresponding variables (DHA for PC1, and 16:1n-7, 18:2n-6, 20:4n-3 and 22:1n-11 for PC2). Besides, NL patterns were less aggregated, possibly reflecting the enrichment diet impact versus *Artemia* FA intrinsic composition. Total lipid FA patterns grouped between PL and NL. Dietary effects on the FA patterns were apparent along PC2, and more marked on the FA profiles of NL, than on those of total lipids and PL in decreasing order. The lipids of unenriched control *Artemia* plotted next to the PL although could be still graphically segregated from them.

4. Discussion

PL have been regarded as most adequate form to present DHA to marine larvae (Gisbert et al., 2005; Wold et al., 2007) since it is an abundant lipid component of larvae's natural copepod diet (Bell et al., 2003; Sargent et al., 1999). Consequently, attempts to deliver HUFA into PL of live preys used in marine larviculture have been made (Harel et al., 1999; Rainuzzo et al., 1994). Marine lecithins, PL-rich materials containing remarkable amounts of HUFA, have been previously used to enrich live preys such as *Artemia* (McEvoy et al., 1996; Monroig et al., 2006). We recently showed that a novel product, the Marine lecithin LC60, was able to simultaneously enhance both PL and HUFA contents of *Artemia* metanauplii, with DHA accounting for up to 13 % of total fatty acids (Guinot et al., 2013). However, the distribution of HUFA, particularly DHA, within the different lipid fractions of *Artemia* metanauplii suggests that these live preys rapidly metabolise the lipids of the enrichment diets during bioencapsulation.

Conversions of the enrichment diets were evidenced by an apparent translocation of DHA from the PL of the enrichment diet Marine lecithin LC60 to NL fractions of metanauplii enriched with this product (Treatment 2). Clearly, DHA accounted for 26.5 % and 6.8 % of total FA in the PL and NL fractions, respectively, in the enrichment diet Marine lecithin LC60. Unexpectedly, the contents of DHA in PL from Treatment 2 *Artemia* (enriched with LC60) were only 1.9 % of total FA, and the NL fractions TAG

and CF accumulated up to 22.2 and 26.0 % DHA, respectively. Despite such undesired conversions might occur, it is worth mentioning that LC60 appears as a highly efficient means for increasing the DHA content of both PL and NL fractions in enriched on-grown *Artemia*, as previously suggested by Harel *et al.* (1999) for nauplii. Non-enriched *Artemia* do not have significant amounts of DHA in their PL, so the presence of PL with ~2 % in live preys can still have a physiological relevance for cultured marine fish larvae. Provided that DHA is virtually absent in both *Artemia* PL and NL (Bell et al., 2003; Conceição et al., 2010), the differential contents of DHA between PL and NL of Treatment 2 *Artemia* cannot respond to a mere dilution effect of the enrichment diet lipids with those of *Artemia.* Otherwise, the ratio PL-DHA vs. NL-DHA existing in the enrichment diet should have been maintained constant in *Artemia* lipids. The translocation of DHA is further evidenced by its presence in transitory storage lipids, such as free fatty acids, monoacylglycerides and diacylglycerides, before it is eventually deposited into TAG.

Previous studies suggested that *Artemia* actively transform lipid components of the enrichment diets during the bioencapsulation process. Retroconversions of DHA to EPA via peroximal β-oxidation (Reddy et al., 2001) were firstly reported by Watanabe et al. (1994) and later demonstrated by Navarro et al. (1999) using radiolabeled FA. Moreover, changes in the positional distribution of FA of dietary TAG were also observed in *Artemia* nauplii enriched with fish oil ethyl esters and TAG (Ando and Narukawa, 2002; Ando and Oomi, 2001; Ando et al., 2004; Shiozaki and Ando, 2004). In line with our observations, DHA contained into fish roe PL was hydrolised and subsequently incorporated into TAG (Shiozaki and Ando, 2004).

The difficulties observed to modify the FA composition of *Artemia* PL might respond to their role as structural molecules in maintenance of cellular homeostasis. Thus, several studies have previously indicated the conservative nature of PL. Coutteau and Mourente (1997) found that absolute concentration of PL remained constant in *Artemia* throughout enrichment with TAG and FA ethyl esters and subsequent starvation. Consequently, no significant amount of DHA was incorporated into PL. Similar results

were obtained when enriching *Artemia* with cod liver oil and n-3 HUFA concentrates (Czesny et al., 1999). Navarro et al. (1999) indicated that radiolabeled C20 and C22 HUFA delivered as ethyl esters and incorporated into TAG, presented low variability among lipid classes in enriched *Artemia* after starvation, while other dietary FA (16:0, 18:0; 18:1, 18:2n-6 and 18:3n-3) exhibited higher mobilisation rates. They suggested that the transferred FA were used as substrates for energy production and for the conservation of membrane phospholipid structure, while HUFA such DHA that do not take part in those functions, were rather stored into NL. Whether such storage fate is a previous step towards its use for energy production remains to be elucidated.

Our PCA results support the above alluded to hypothesis by which the FA composition of PL from *Artemia* cannot be substantially altered. PL, associated to 18:0, 18:4n-3, 18:2n-6, 20:0, 20:3n-3 and 22:0, remain remarkably constant between treatments. In addition, FA profiles from PL were similar to those from the TL and PL fractions of non-enriched metanauplii, indicating a conservative profile of PL. NL distribution in the score plot show a bigger dispersion and they are highly associated to DHA (present in both Marine lecithin LC60 and Easy DHA Selco) and LA (present in SL). PCA shows a marked effect of enrichment diet FA on NL, thus indicating a less conservative nature of NL in comparison to PL and possibly reflecting the metabolic activity that the incorporated FA are being submitted to. Importantly, PCA revealed that TAG appear to be a predominant metabolic fate of the DHA supplied through enrichment diet. The hydrolysis of DHA from dietary PL and subsequent re-esterification into NL might be a reflection of the apparent non-essential nature of DHA for *Artemia* (Navarro et al., 1992)

In addition to non-essential FA such as DHA, other essential FA also accumulate in NL of *Artemia* when supplied in excessive amounts. That is the case of the LA (18:2n-6), the most abundant FA of soya lecithin used in Treatment 1 and a constitutive FA of *Artemia franciscana* (Léger et al., 1986). LA contained in the soya lecithin, rather than remaining esterified as PL for its direct depostion in *Artemia*, was accumulated into TAG or else converted into other FA (Ito and Simpson, 1996; Schauer and Simpson,

1985). Several enzymes including phospholipases, acyl transferases and phosphatases might be responsible for the translocations of FA between PL and TAG occurring in different metabolic pathways such as the glycerol phosphate pathway (phospholipid and TAG metabolism interlink) and Lands' cycle (Bankaitis, 2009; Gurr and Harwood, 1991). Possibly, the study of the metabolic mechanisms underpinning the undesired conversions during *Artemia* enrichment, as well as the identification of specific enzymatic activities controlling them, might open a promising area of research in the future. Importantly for aquaculture, such investigations would help us to optimise the bioencapsulation of essential lipid components into *Artemia*.

In summary, on-grown *Artemia* actively convert FA delivered as PL to TAG, with DHA being preferentially transferred from dietary PL to TAG. The assimilation and metabolism of the FA contained in enrichment diets for *Artemia* involves an important handicap for bioencapsulation of essential lipid compounds and consequently for their use as live preys in larviculture. Further studies are required to elucidate the causes of such undesired conversions so that practical procedures to optimise the delivery of HUFA-rich PL into live preys can be implemented.

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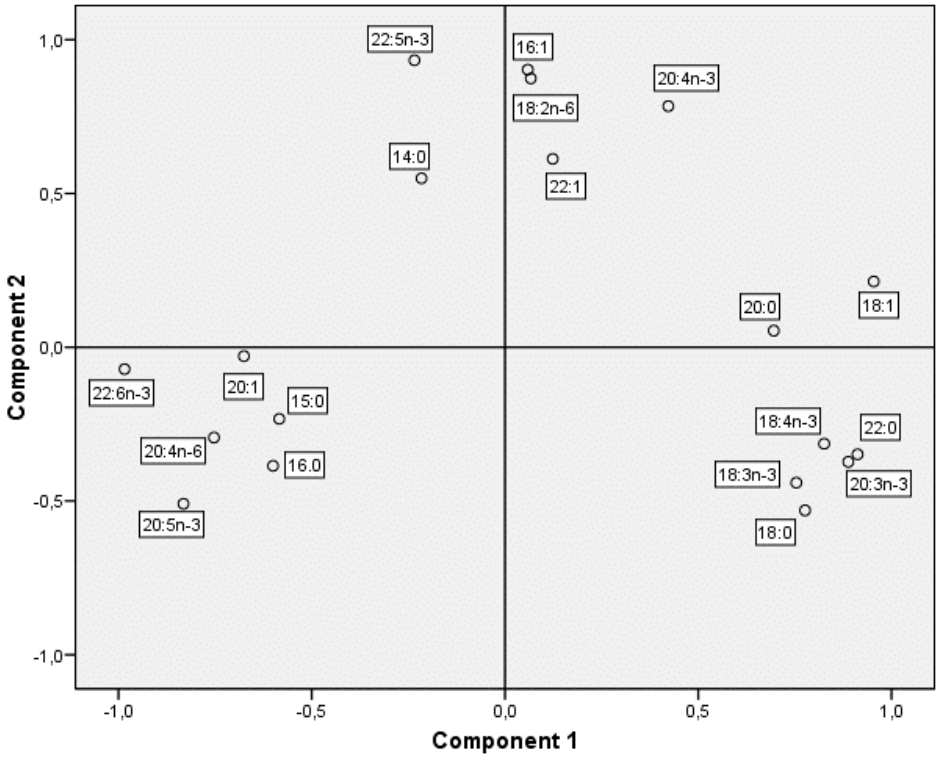
Figure 1. A) Component plot of principal component analysis (PCA) of selected fatty acids from total, neutral and polar lipids of *Artemia* metanauplii enriched with Soya Lecithin mixed with Easy DHA Selco and Marine Lecithin. **B)** Factor score plot of PCA of selected fatty acids from total, neutral and polar lipids of *Artemia* metanauplii enriched with Soya lecithin mixed with Easy DHA Selco (Treatment 1) or Marine lecithin (Treatment 2). Grouping is based on the lipid classes (total, polar or neutral) fatty acids of enriched *Artemia* metanauplii.

A)



B)

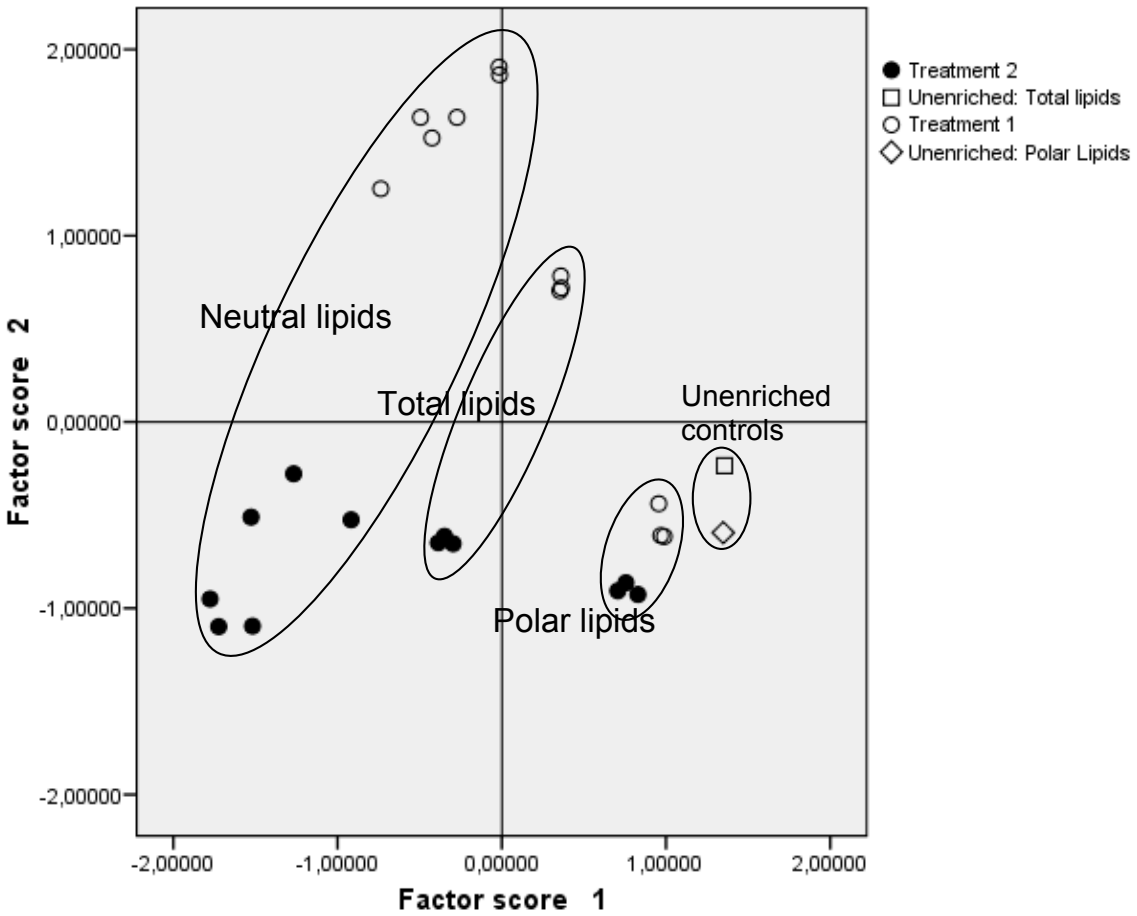

Treatment 2
Unenriched: Total lipids
Treatment 1
Unenriched: Polar Lipids
Neutral lipids
Total lipids
Unenriched controls
Polar lipids
Factor score 2
Factor score 1
2,00000
1,00000
0,00000
-1,00000
-2,00000
-2,00000
-1,00000
0,00000
1,00000
2,00000

Table 1. Selected fatty acid contents (percentage of total fatty acids) of total lipids, polar lipids (PL) and neutral lipids (NL) of products (Soya lecithin, Easy DHA Selco and Marine lecithin LC60) used in enrichment diet preparation.

Fatty acid	Soya lecithin			Easy DHA Selco			Marine lecithin LC60		
	Total lipids	PL	NL	Total lipids	PL	NL	Total lipids	PL	NL
14:0	0.1	0.1	0.6	3.2	11.2	4.6	1.5	1.4	3.7
15:0	0.1	0.1	0.4	0.5	0.5	0.7	0.6	0.6	0.9
16:0	21.7	22.8	15.9	12.4	21.8	17.7	31.2	33.8	22.4
16:1n-7	0.1	0.1	ND	4.5	4.5	6.7	0.5	0.1	2.8
16:2	0.1	0.2	2.0	0.2	ND	0.3	0.9	0.9	2.1
18:0	3.7	3.8	5.8	3.8	5.4	4.2	4.1	4.1	7.1
18:1	15.2	15.6	28.4	13.8	14.9	16.7	2.1	3.8	27.1
18:2n-6	52.0	51.5	31.6	4.7	19.0	5.1	0.5	0.2	4.5
18:3n-3	4.1	4.1	2.9	1.1	2.4	1.2	ND	0.1	3.4
18:4n-3	ND	0.0	ND	1.6	1.4	1.5	0.1	0.1	1.2
20:0	0.1	0.2	0.5	0.5	0.9	0.2	ND	0.1	ND
20:1n-9	ND	0.1	ND	2.5	ND	2.0	4.9	4.5	4.9
20:4n-6	ND	ND	ND	1.5	0.7	1.4	1.8	1.9	0.8
20:5n-3	ND	ND	ND	17.2	5.7	9.4	13.7	13.4	6.8
22:0	0.4	0.4	ND	0.1	0.3	0.1	ND	0.1	ND
22:1n-11	ND	ND	ND	1.3	ND	1.1	0.7	0.8	0.7
22:6n-3	ND	0.0	ND	18.3	4.3	16.1	33.0	31.8	8.6

ND, not detected.

Table 2. Selected fatty acid content (percentage of total fatty acids) of total lipids, polar lipids (PL) and neutral lipids (NL) fractions from Treatment 1 *Artemia* metanauplii. NL include two distinct fractions, one corresponding to triacylglycerides (TAG) and another to the combined fraction (CF) of monoacylglycerides, diacylglycerides and free fatty acids. Data represent mean ± standard deviation (n=3).

	Total lipids	PL	NL	
			TAG	CF
14:0	0.9 ± 0.0	0.4 ± 0.0	1.0 ± 0.2	0.8 ± 0.1
15:0	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	1.2 ± 1.5
16:0	14.2 ± 0.2	12.9 ± 0.4	13.6 ± 0.90	11.0 ± 0.7
16:1n-7	3.6 ± 0.1	2.5 ± 0.5	4.4 ± 0.2	5.3 ± 1.4
16:2	0.6 ± 0.0	1.1 ± 0.0	1.0 ± 0.1	1.4 ± 0.8
16:3	0.5 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	ND
18:0	7.2 ± 0.1	10.1 ± 0.1	5.1 ± 0.4	3.3 ± 0.3
18:1	26.2 ± 0.5	30.1 ± 0.6	20.4 ± 0.7	25.4 ± 1.0
18:2n-6	9.1 ± 0.9	6.8 ± 0.4	10.9 ± 1.1	14.1 ± 1.6
18:3n-3	11.2 ± 0.5	16.3 ± 0.3	7.1 ± 1.0	9.9 ± 0.5
18:4n-3	2.2 ± 0.1	3.0 ± 0.1	1.0 ± 0.8	1.7 ± 0.1
20:0	0.2 ± 0.0	0.2 ± 0.0	2.4 ± 0.1	1.4 ± 0.2
20:1n-9	1.8 ± 0.0	1.3 ± 0.0	ND	ND
20:4n-6	1.5 ± 0.1	1.7 ± 0.0	1.4 ± 0.1	1.9 ± 0.1
20:3n-3	0.4 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	ND
20:4n-3	0.4 ± 0.0	0.2 ± 0.0	0.5 ± 0.0	0.5 ± 0.1
20:5n-3	7.6 ± 0.3	7.9 ± 0.2	7.1 ± 0.4	9.7 ± 0.8
22:0	0.4 ± 0.0	0.8 ± 0.1	1.0 ± 0.8	ND
22:1n-11	0.5 ± 0.0	0.1 ± 0.1	0.2 ± 0.0	0.4 ± 0.1
22:5n-3	0.5 ± 0.0	0.1 ± 0.0	1.0 ± 0.1	0.7 ± 0.1
22:6n-3	5.8 ± 0.3	0.8 ± 0.1	10.4 ± 0.7	12.7 ± 1.2

ND, not detected.

Table 3. Selected fatty acid content (percentage of total fatty acids) of total lipids, polar lipids (PL) and neutral lipids (NL) fractions from Treatment 2 *Artemia* metanauplii. NL include two distinct fractions, one corresponding to triacylglycerides (TAG) and another to the combined fraction (CF) of monoacylglycerides, diacylglycerides and free fatty acids. Data represent mean ± standard deviation (n=3).

	Total lipids	PL	NL	
			TAG	CF
14:0	0.7 ± 0.0	0.4 ± 0.0	0.7 ± 0.1	0.7 ± 0.2
150	0.4 ± 0.0	0.3 ± 0.0	0.4 ± 0.0	3.5 ± 0.6
16:0	19.4 ± 0.4	15.6 ± 0.5	20.6 ± 1.8	16.3 ± 2.8
16:1n-7	0.9 ± 0.1	1.1 ± 0.1	1.2 ± 0.2	ND
16:2	0.4 ± 0.0	1.1 ± 0.0	1.2 ± 0.6	1.7 ± 0.1
16:3	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	ND
18:0	7.6 ± 0.1	10.3 ± 0.7	6.1 ± 3.0	4.8 ± 1.0
18:1	17.8 ± 0.1	25.9 ± 1.9	10.2 ± 1.0	12.3 ± 3.8
18:2n-6	2.8 ± 0.1	4.3 ± 0.2	1.5 ± 0.3	2.8 ± 0.6
18:3n-3	11.2 ± 0.5	16.5 ± 0.5	6.6 ± 1.5	9.1 ± 1.3
18:4n-3	2.0 ± 0.1	3.0 ± 0.2	1.1 ± 0.2	1.3 ± 0.2
20:0	0.2 ± 0.0	0.2 ± 0.0	3.9 ± 0.4	2.1 ± 0.1
20:1n-9	2.4 ± 0.0	1.5 ± 0.1	ND	ND
20:4n-6	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.3	2.4 ± 0.1
20:3n-3	0.4 ± 0.0	0.6 ± 0.1	0.2 ± 0.0	ND
20:4n-3	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	ND
20:5n-3	12.6 ± 0.4	10.2 ± 0.7	14.7 ± 2.8	18.3 ± 1.8
22:0	0.4 ± 0.0	0.8 ± 0.2	ND	ND
22:1n-11	0.2 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	ND
22:5n-3	0.2 ± 0.0	0.0 ± 0.0	0.3 ± 0.10	ND
22:6n-3	13.1 ± 0.3	1.9 ± 0.1	22.2 ± 4.6	26.0 ± 2.3

ND, not detected.

Table 4. Selected fatty acid content (percentage of total fatty acids) of total lipids, polar lipids (PL) and neutral lipids (NL) fractions from unenriched *Artemia* metanauplii. NL include two distinct fractions, one corresponding to triacylglycerides (TAG) and another to the combined fraction (CF) of monoacylglycerides, diacylglycerides and free fatty acids.

	Total lipids	PL	NL	
			TAG	CF
14:0	1.7	0.3	1.7	
15:0	0.7	0.3	7.1	
16:0	9.4	9.9	13.5	27.8
16:1n-7	1.0	1.2	3.6	
16:2	0.1	1.1	0.8	
16:3	0.4	0.4	ND	
18:0	9.8	10.6	5.2	18.4
18:1	29.9	32.6	16.9	33.7
18:2n-6	3.0	3.2	4.6	
18:3n-3	13.4	14.2	18.9	20.1
18:4n-3	4.8	5.2	2.6	
20:0	0.2	0.1	2.9	
20:1n-9	1.1	1.1	ND	
20:4n-6	1.0	1.0	ND	
20:3n-3	0.7	0.7	0.8	
20:4n-3	0.5	0.4	ND	
20:5n-3	7.0	7.4	1.4	
22:0	0.9	0.8	ND	
22:1n-11	ND	0.1	ND	
22:5n-3	ND	ND	ND	
22:6n-3	ND	ND	ND	

ND, not detected.